

Amendments to the Specification and Abstract

In the Title:

Please amend the title as follows: ~~METHOD FOR AUTOMATIC PRODUCTION OF AUTOMATICALLY GENERATING LASER CUTTING LINES IN LASER MICRO-~~
DISSECTION MICRODISSECTION PROCESSES

In the Specification:

Please replace paragraph [0001] with the following rewritten paragraph:

[0001] The present invention relates to a laser microdissection method ~~having the features set forth in the definition of the species in claim 1. Advantageous embodiments are derived from the dependent claims~~ in which a nominal cutting line is marked for an object to be cut out from a microscopic specimen, and the object is subsequently cut out in response to a relative motion between a laser beam and the specimen.

Before paragraph [0002] please insert the following new heading:

BACKGROUND

Before paragraph [0005] please insert the following new heading:

SUMMARY OF THE INVENTION

Please replace paragraph [0005] with the following rewritten paragraph:

[0005] It is, therefore, ~~the~~ an object of the present invention to ~~devise~~ provide a laser microdissection method which will permit a high specimen throughput in a largely error-free process.

Please replace paragraph [0006] with the following rewritten paragraph:

[0006] ~~This objective is achieved by a laser microdissection method of the species having the characterizing features set forth in claim 1.~~ The present invention provides a laser microdissection method in which a nominal cutting line is marked for an object to be cut out from a microscopic specimen, and the object is subsequently cut out in response to a relative motion between a laser beam and the specimen. In the method, an electronic image (grayscale

image or color image) of at least one image detail of the specimen is captured. Image analysis is subsequently used to process the image detail and to determine the object to be cut out. The nominal cutting line around the object to be cut out is subsequently automatically defined. In response to a user command, the object may then be cut out along the nominal cutting line by a laser beam.

Before paragraph [0016], please insert the following new heading:

BRIEF DESCRIPTION OF THE DRAWINGS

Please replace paragraph [0016] with the following rewritten paragraph:

[0016] The present invention is described in greater detail in the following based on exemplary embodiments with reference to the schematic ~~drawing, whose figures~~ drawings, which show:

Before paragraph [0025], please insert the following new heading:

DETAILED DESCRIPTION

Please replace paragraph [0028] with the following rewritten paragraph:

[0028] Object features may be ascertained by performing image analysis calculations. The complete functional sequence, including the determination of the object features, may be broken down into different incremental steps. The flow chart illustrating one advantageous embodiment of the method is presented in FIG. 1.2. It includes the following steps ~~1 through~~ & 101 through 108.

Please replace paragraph [0029] with the following rewritten paragraph:

[0029] ~~1-~~ Step 101: Image acquisition:

The image is first acquired using a suitable device, for example a microscope having an adapted camera; the camera may be an analog or a digital camera. Depending on the type of objects to be classified, a color or a grayscale camera may be used.

Please replace paragraph [0030] with the following rewritten paragraph:

[0030] ~~2- Step 102:~~ Shading Correction:

Shading correction may then be automatically applied to this image; i.e., this step is optional. This process takes into account that the image quality is already distorted by the illumination due to an inhomogeneous illumination of the image field. This problem is overcome in the present case by the automatic use of a shading image which is automatically or manually captured beforehand and stored. In the process, the specimen slide stage is moved to a so-called empty position which is characterized in that, at this location, the system is able to acquire an undistorted image of the illumination distribution by using an empty specimen slide. This image is undistorted since there is no biological material on the specimen slide between the objective and the illumination source. This correction image is then applied later to all images captured under the same optical conditions, in order to automatically correct the shading influence caused by the inhomogeneity of the specimen illumination.

Please replace paragraph [0031] with the following rewritten paragraph:

[0031] ~~3- Step 103:~~ Grayscale Image Processing:

In accordance with the schematic representation in FIG. 1.2, the "grayscale value processing" follows at this point. The shading correction referred to above may also already be understood to be grayscale value processing. In this step, minor artifacts in the image are first removed using grayscale morphology.

Please replace paragraph [0032] with the following rewritten paragraph:

[0032] ~~4- Step 104:~~ Threshold Value and Transition to the Binary Image:

Various image analysis methods are known for threshold value determination. To obtain a most rugged method possible, the optimal threshold value for the transition from the grayscale image to the binary image, usually referred to as segmentation, may preferably be ascertained using a so-called entropy maximization approach. The idea underlying entropy maximization is for a threshold value to be determined in the grayscale value histogram of the image in such a way that the binary image derived by applying this threshold value exhibits a maximum possible entropy. As is known, for example, from H. Rohling, "Einführung in die Informations- und Codierungstheorie" [An Introduction to Information and Encoding Theory], Teubner Publishers 1995, the entropy content is a measure of the information content of an image. Thus, by determining the threshold value using the entropy

maximization approach, a binary image is obtained having a maximum possible information content.

Please replace paragraph [0035] with the following rewritten paragraph:

[0035] ~~5-~~ Step 105: Binary Image Processing:

In accordance with FIG. 1.2, the binary image processing follows at this point as a further step. In this case, minor artifacts (individual pixels, small pixel clusters, etc.) are removed from the image. In accordance with the present invention, the purpose of this procedure is to eliminate small objects having a diameter that is too small for the laser cutting process, before the cutting operation. In this context, the value below which an object is considered to be too small for the laser cutting process, is settable. In this connection, the morphology known from the image analysis processes may be used. Image analysis morphology is discussed in detail in Sierra, J., "Image Analysis and Mathematical Morphology", Academic Press, 1988. In the described method, erosion is employed in particular as a special morphological filter for image processing. By selecting the magnitude of the morphological operator (SE = structuring element, term from mathematical morphology) or, however, in equivalent manner, by selecting the number of cycles in which an SE of a specific size is applied to the binary image, the user may set the particle sizes that are to be excluded before the laser cutting. Moreover, by utilizing the possibilities offered by image analysis morphology, it is also possible for very specific shapes, thus not only object sizes, but also objects having specific shapes, to be filtered out from the image. Thus, for example, lanceolated small objects may be successfully ignored, while small round objects are delivered to the cutting process, unfiltered.

Please replace paragraph [0036] with the following rewritten paragraph:

[0036] ~~6-~~ Step 106: Segmentation and Determination of the Object Features:

In this analysis step, the object features of each segmented object are first determined (so-called feature extraction). The features utilized for the object classification are determined from the binary image and subsequently termed classification features. Classifiable features include all features which are presently measurable by image analysis or which are derivable from a priori knowledge, as well as any given linear combination of the parameters. Examples of features are surface area, convex surface, equivalent diameter, length, width,

angle, orientation, roundness, length-width ratio, bulging, color values in RGB metric or any other color metric, curve length, curve width, horizontal and vertical projection, texture, energy, etc.

Please replace paragraph [0039] with the following rewritten paragraph:

[0039] ~~7. Step 107:~~ Object Classification:

In the next step, the extracted object features are compared to the predefined classification features. To that end, the combinations of measured object features of the objects to be cut out using the laser are checked for conformity with the values of the classification features. In this manner, the desired objects to be cut out are differentiated from those that are not desired. Since a number of characterizing features were specified for the desired objects of the same type, and since other features were specified for objects of another type, all objects may be uniquely assigned to one such type or be classified as waste, thus as unusable material. The unusable objects are then separated out and, thus, also not cut out.

Please replace paragraph [0047] with the following rewritten paragraph:

[0047] ~~8. Step 108:~~ Automatic Determination of the Cutting Lines for the Laser:

Once the objects designated for microdissection are identified, the cutting operation is prepared in the last step in accordance with FIG. 1.2. The object contour of each identified object is first determined through image analysis. This object contour is indicated on the specimen in the xy coordinates where the laser is to perform the cutting operation. In this manner, a cutting line is automatically determined by the system.

Please replace paragraph [0048] with the following rewritten paragraph:

[0048] Prior to the automatic cutting line determination, however, the identified objects may still be selectively subjected to further process steps, as described in the following. For example, groups of objects situated closely together are clustered in the image, i.e., combined into one shared object group to be cut out, as shown in FIG. 1.3. Here, four objects 34 disposed in close proximity to one another form one cluster 35. A shared, externally surrounding outer cutting line 36 is then defined for this cluster. The laser is thus prevented in accordance with the present invention from "slicing through" adjacent specimen objects during the cutting operation, as these objects are too closely proximate to the object actively

being cut out (compare FIG. 1.3). Here as well, the morphology may be employed: In the present exemplary embodiment, a clustering is achieved by morphological closing, in image analysis, n-times closing meaning the sequential execution of n dilations, followed by n erosions. This principle is known from Schnaider; see [http://www.zgdv.de/zgdv/departments/z2/Z2Staff/schnaide/local images/ME200 3-02.pdf](http://www.zgdv.de/zgdv/departments/z2/Z2Staff/schnaide/local%20images/ME200%203-02.pdf).

Please replace paragraph [0066] with the following rewritten paragraph:

[0066] The outer contour of each image object, which does not have any more holes, may subsequently be easily defined. To this end, it must be ascertained that every pixel which is entirely situated within an object, is completely surrounded by object pixels of the same grayscale value. Therefore, any pixel that is not completely belonging surrounded by object pixels, must belong to the outer boundary of the object, if the object does not have any holes. This criterion is checked for each pixel, and the boundary pixel is determined in this manner.

Please replace paragraph [0070] with the following rewritten paragraph:

[0070] Since each object contour is completely measured off in steps, or incrementally scanned, starting from the particular FCP, a Freeman code in the form of a number chain is obtained, which fully describes the contour of the particular object. Since, in addition, the coordinates of the FCP are stored for each object, all of the objects in the image are fully describable in terms of contour and position. FIG. 1.7c) shows the corresponding notation of the Freeman code for the boundary pixels of the object shown in FIG. 1.7b). Using this encoding method, a list encompassing the contour data on all of the objects is generated as an interim result. It includes the particular position of the FCP, starting at which an object contour must be described.

	FCP x	FCP y	Freeman code describes the outer laser cutting line of the object
object 1	x ₁	y ₁	2 3 3 3 5 6 3 3 4 5 6 7 3 0 0 0 ...
object 2	x ₂	y ₂	4 2 2 3 3 4 6 7 7 7 6 0 0 2 3 4 5 ...
object 3	x ₃	y ₃	6 6 7 3 3 3 2 2 2 1 1 7 6 6 0 0 3 3 3 ...
object 4	x ₄	y ₄	3 3 6 5 2 2 1 0 0 6 5 5 ...
...			
object n	x _n	y _n	2 2 3 5 6 6 6 7 6 5 5 3 2 1 1 0 0 0 ...

Table 1.5: (Example) Determining the Laser Cutting Code by Applying a Contour Analysis Method

Please replace paragraph [0091] with the following rewritten paragraph:

[0091] In the following, it is assumed that the laser cutting line is already known by having been previously defined (automatically or manually). The laser cutting line is composed of a series of points P_i P_i having coordinates (x_i, y_i) and is completely described by the same. In the case of scaling, it holds for every point P that

$$\begin{aligned}x_i' &= sx \cdot x_i \\y_i' &= sy \cdot y_i\end{aligned}$$

sx and sy being scaling factors. In vector notation, this is described as

$$P_i = \begin{pmatrix} x_i \\ y_i \end{pmatrix} \quad S = \begin{pmatrix} sx & 0 \\ 0 & sy \end{pmatrix} \quad P_i' = \begin{pmatrix} x_i' \\ y_i' \end{pmatrix},$$

P_i being the i -th original point and P_i' the i -th scaled point of the laser cutting line. Thus, the scaling equation is expressed as

$$P_i' = S \cdot P_i$$

for all points i from the interval $[0, k]$ of the laser cutting line. In this procedure, the laser cutting line is scaled in such a way that the object is able to be cut out at a safe clearance distance. The distances, by which the individual contour points are spaced apart and which are a consequence of this type of scaling, are compensated in that the laser beam cuts in straight lines from point P_i to point P_{i+1} . Thus, given substantial enlargements, the continuously extending laser cutting line approximates a polygon in that individual points P_i are joined by straight segments of the laser cutting line.

Please replace paragraph [0115] with the following rewritten paragraph:

[0115] In response to control of laser scanning device 22, laser beam 7 emerges at the output thereof at various deflection angles. In the process, by varying the deflection angle, laser beam 7 may be directed to any given positions on specimen 4 which are located within the field of view of objective 10 9.

Please replace paragraph [0139] with the following rewritten paragraph:

[0139] Objects which touch the image edge are not able to be completely cut out by laser. Moreover, due to the incomplete form, there is the danger of erroneous classification. Therefore, such objects are ignored when necessary, by checking during the object identification process to determine whether an object is touching the image edge or not. Then, in dependence upon the analysis result, the object is may be blocked out prior to the further process steps or.

Please delete paragraph [00167] .